

PARAGONIMUS, ITS LIFE HISTORY AND
DISTRIBUTION IN NORTH AMERICA

AND ITS
TAXONOMY (TREMATODA: TROGLOTREMATIDAE)

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PARAGONIMUS, ITS LIFE HISTORY AND DISTRIBUTION IN
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Introduction.

Paragonimus, the lung fluke of mammals, has long been recognized as one of the important parasites of man in Asia, especially in Japan, Korea and Formosa, where much information has been secured, chiefly by medical men, on its taxonomic and biological as well as its medical aspects. In the western hemisphere, information concerning *Paragonimus* is meager and reports have dealt mainly with the finding of the worm in various hosts and localities and with morphology and taxonomy. Although this worm has been reported but a few times as a human parasite in the Americas, it must be looked upon as a potential human parasite which awaits only a change in the

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diet of human beings to assume considerable importance. It is now of some significance as a parasite of domestic animals and certain valuable fur bearers and thus is of interest to veterinarians and conservationists.

The present research started in the fall of 1929 when a cat was secured from Platt, a small village on the outskirts of Ann Arbor, Michigan, to use as an experimental host for a larval cestode. That experiment failed, but the cat was discovered to be infected with *Paragonimus*. Realization of the need for a thorough study in North America of this important parasite and recognition of the fact that the stages in its life history must be present in this locality gave impetus to this investigation which is concerned with four phases. These are: (1) the determination of the life history of *Paragonimus* in North America, (2) the description of the stages in this life history, (3) the taxonomy of the genus and the establishment of a basis for differentiating the species, if possible, and (4) the ascertaining of its distribution in North America so far as practicable.

Historical. Those interested in a complete historical account of *Paragonimus* are referred to Ward and Hirsch (1915) and to Khaw (1930).

Technique. Cercariae, metacercariae, miracidia, sporocysts, and first and second generation rediae were studied alive and with the aid of *intra vitam* stains. Twenty-eight stains were used, of which neutral red, brilliant cresyl blue, malachite green, Nile blue sulphate and rhodamine G were found to be the most satisfactory. Of the above named stains, Nile blue sulphate was a 0.2 per cent aqueous solution while the remainder were saturated aqueous solutions. When applied, these were diluted considerably depending upon the subject. All the stages in the life cycle listed above and the adult worms were fixed either in Gilson's or Bouin's fluids, stained *in toto* with pararcarmine and haematoxylin stains and mounted in balsam or damar. Cercariae and metacercariae were also sectioned.

The hearts of crayfishes were examined for metacercariae by removing the carapace over the pericardial region. In living crayfishes, the metacercariae are recognizable to the naked eye because of the contrast that their white excretory bladders presents to the yellowish host tissue. The hearts of preserved crayfishes were removed and cleared in liquefied phenol to reveal the presence of cysts. In the study on distribution, all metacercariae concerned were observed under a compound microscope.

Other special methods are described in appropriate places in this report.

Definitive hosts.

Since Ward first reported *Paragonimus* from the cat in North America, it has been recorded on this continent from the pig, dog, mink, muskrat, wildcat, goat and man (see under distribution). Examination of mink by Ameel (1931a) and by Wallace (1931) indicates that the mink, *Mustela vison mink*, is the usual definitive host. During the 1930 trapping season, 563 mink secured from trappers and fur buyers in Michigan and northern Ohio were examined and 17 per cent were reported as having been infected. This, however, was an error, 15 per cent being the actual percentage. Later, an additional 25 mink were examined and 11 of these were infected, making a total of 588 mink with a 16 per cent infection. Wallace's material came from fur farms in Minnesota. He examined 84 carcasses and the feces from 150 living animals and found that 7 per cent were infected. Ameel (1932a) reported the presence of *Paragonimus* in muskrats, *Ondatra zibethica*, from the Muskegon and Manistee marshes in Michigan. Of 79 carcasses examined, 10 (12 per cent) were infected. Subsequent collections from the same areas added 27 carcasses, of which 8 were infected, bringing the percentage of infected animals to 16 per cent. No lung flukes were found in 14 muskrats from two localities near Ann Arbor where crayfishes are numerous and heavily infected. They had not been found in 249 muskrats, from numerous localities in the southern peninsula of Michigan, examined previous to 1929.

Since the crayfish is a favorite item of diet for raccoons, these animals are exposed to considerable numbers of metacercariae wherever *Paragonimus* occurs. However, no trace of the parasite was found in 308 carcasses of raccoons secured from areas within the range where *Paragonimus* was known to exist, indicating that the raccoon possesses an immunity to the lung fluke. This conclusion was strikingly confirmed by a laboratory experiment. Three raccoons, aged several years, 8 months, and 8 weeks respectively, were fed large numbers of metacercariae. Upon examination, all were negative and only the eight-month old animal bore any evidence of possible effects of infection in the form of adhesions of the lungs to the pleural wall.

Infected opossums have never been found although they do occasionally eat crayfishes. Dearborn (1932) reports that 3.52 per cent of the food (measured volumetrically) eaten by 40 opossums, the feces of which he examined, consisted of these crustaceans. No lung flukes were found in 109 animals from endemic areas and an attempt to infect an opossum in the laboratory failed though it was

given a large number of viable cysts, indicating that this mammal also is probably immune. There may be some significance in the fact that the families to which the raccoon and opossum belong exist only in the Americas. The remaining hosts examined, consisting of 58 weasels, one badger and three otters, were uninfected.

A large number of white rats and domestic cats as well as 3 muskrats and one dog were used as experimental hosts in the course of this investigation. The cat was the most satisfactory experimental host. The white rat did not prove to be a good host since the adult worms in many cases disappear from the cysts in the lungs within two to three months after the ingestion of the metacercariae. The longest time any fluke remained in the lungs was 7 months. Many of the metacercariae upon entering the pleural cavity failed to enter the lungs and remained free in the cavity for some months. Others lingered in the abdominal cavity for long periods of time. In both cases, they increased in size two to four times and failed to develop further. No worms were recovered from the abdominal cavity after 4½ months subsequent to infection but they were recovered from the pleural cavity of rats up to the end of the experiment, 8½ months following infection.

First intermediate hosts.

In the summer of 1931, the writer (Ameel 1932b), found the opereulate snail, *Pomatiopsis lapidaria* Say (plate VI, fig. 99), to be the first intermediate host of the North American lung fluke.

Habitat and habits. The habitat preference of *P. lapidaria* has been the subject of considerable controversy but it is generally considered to be amphibious in habit. I have observed the habits of this snail for several years while collecting them for use in this study. Though the snails were very abundant in two localities where collections were made, none were observed on the bottom of the adjacent streams. However, a heavy rain in the spring of 1933 caused the streams to overflow and to cover the habitat of the snails to a depth of two and one-half to three feet. The water gradually receded over a period of a week during which time the snails remained submerged and made no effort to leave the water.

P. lapidaria is more or less nocturnal. On bright days, the snails remain hidden under the leaves and other cover but expose themselves on dull days or in well shaded situations provided their cover is well saturated with moisture. Several visits were made on warm humid evenings to a locality where the snails occurred in abundance and on

each occasion numerous snails were found in exposed situations. Many were upon the ground, some were partially or completely submerged in small pools of water and others had climbed up the standing vegetation to a height of 8 inches or more. When the place was visited on the days following these observations, the snails had taken to cover.

Apparently, if adult snails are in good condition, they can withstand long periods of desiccation. On several occasions, a large number of snails survived a two months' period of desiccation in the laboratory.

The most satisfactory method for keeping these snails alive in the laboratory was to place them in uncovered shallow dishes provided with damp soil and decomposing leaves.

Second intermediate hosts.

Representatives of practically every subdivision of the crayfish genus *Cambarus* were found to serve as secondary intermediate hosts in North America. Metacercariae were found in the following species identified through the courtesy of Dr. E. P. Creaser, Museum of Zoology, University of Michigan: *C. propinquus* Girard, *C. virilis* Hagen, *C. robustus* Girard, *C. versutus* Hagen, *C. diogenes* Girard, *C. blandingii acutus* Girard, *C. sloanei* Bundy, *C. rusticus* Girard, *C. obscurus* Hagen and *C. creolanus* Creaser. *C. immumis* Hagen secured from a large pond near Ann Arbor was used in infection experiments.

Small streams appear to be the most favorable habitats for the maintenance of the life cycle of *Paragonimus*. Slow moving streams 20 to 30 feet or less in width provided the greatest numbers of infected crayfishes as well as the most highly infected, while only rarely, if at all, was an infected crayfish collected in the larger streams, although large collections were made from them.

The possibility that the fresh water shrimp, *Palaemonetes exilipes* Stimpson, may serve as a second intermediate host of *Paragonimus* was considered, for this decapod is often found in the same habitat with the crayfish, particularly in the southern states. Specimens taken from a stream in Michigan inhabited by infected crayfishes were examined for metacercariae but none were found. An attempt to infect a small number experimentally by exposing them to a large number of cercariae failed.

Egg.

Measurements. Many investigators have emphasized the size of the egg of *Paragonimus* as a means of species differentiation. To show how inadequate and variable this character is, measurements were taken of 100 eggs, 25 from the fresh feces of 4 different species of hosts. The eggs, kept free from pressure, measured as follows: mink, 0.075–0.088 mm. by 0.053–0.060 mm. (av., 0.083 by 0.056 mm.); muskrat, 0.075–0.105 mm. by 0.053–0.063 mm. (av., 0.089 by 0.057 mm.); cat, 0.083–0.100 mm. by 0.055–0.065 mm. (av., 0.090 by 0.058 mm.); white rat, 0.073–0.090 mm. by 0.050–0.058 mm. (av., 0.079 by 0.055 mm.); average of the 100 eggs, 0.085 by 0.056 mm. Thus it appears that eggs known to be of the same species and measured under the same conditions may show considerable variation in size. The range in size is even greater if account is also taken of egg measurements of the American lung fluke by Ward (1894b: 356) in the cat, 0.096–0.118 mm. by 0.048–0.050 mm. (av., 0.102 by 0.053 mm.) and by Feldman and Essex (1929: 204), in lung sections of a cat, 0.088–0.104 mm. by 0.052–0.062 mm. (av., 0.093 by 0.057 mm.).

Measurements of the eggs of the Oriental lung fluke have been given as follows: Kerbert (1878: 273) from the tiger, 0.075 by 0.04 mm.; Katsurada (1900: 509), eggs from human sputum, 0.0875–0.1025 mm. by 0.0525–0.0675 mm. (av., 0.0935 by 0.057 mm.); Kobayashi (1918: 110), from human sputum, 0.082–0.086 mm. by 0.046–0.05 mm.; Vevers (1923: 12) tiger, 0.085 by 0.055 mm., mungoose, 0.075 by 0.048 mm. It may be noted that the upper limit of size of eggs of the American lung fluke is greater than that of the Oriental form. Thus, the size of the egg seems to have little value as a specific character. It must be noted, however, that some of the variation in size of material of this nature is due to the differences in technique involved in handling it. In the present study, care is taken to state definitely the condition of the material at the time of measurement.

Description. The egg shell is yellowish-brown in color and possesses, at one end, an operculum surrounded by a distinctly thickened collar, while the opposite end is more or less thickened and is occasionally provided with a nodule of varied form. Eggs recovered from the feces and lung cysts are in the single-cell stage (plate I, figs. 3 and 4). The ovum, usually centrally located, is barely visible through the coarsely granular yolk cells which surround it. The yolk cells are large and ordinarily number from 5 to 10. Their outlines are fairly distinct in fresh eggs but the cells soon lose their identity especially after incubation begins.

Sectioned eggs. The contents of the eggs are best observed in thin sections of adult worms stained in Flemming's triple stain or iron hematoxylin. Eggs in the uterus near the ovary contain an ovum which is undergoing maturation and is terminally located in relation to the mass of yolk cells (plate I, fig. 7). In these eggs and in those slightly more developed which have been stained in Flemming's triple stain, granules of shell material within the yolk cells and arranged upon the inner surface of the shell are easily identified by their deep red color. In older eggs, all of the shell material has been released from the vitelline cells and is incorporated in the shell. The ova have usually taken up their normal central position among the yolk cells (plate I, figs. 5, 6 and 11), but occasionally an ovum is found occupying a peripheral position even in eggs in the metraterm (plate I, figs. 9 and 10). The yolk cells consist of very granular cytoplasm and a conspicuous nucleus with a large nucleolus. The ovum is easily distinguished from the yolk cells by its smaller size and the finer composition of its cytoplasm. Eggs in the pronuclear stage (plate I, figs. 5 and 11) occur in all parts of the uterus, excluding the portion adjacent to the ovary. The pronuclei possess smaller nucleoli than those of the yolk cells. There is no positive evidence anywhere of a fusion of the nuclei which probably takes place after the eggs are extruded from the worm.

Development. All eggs used for development of miracidia were procured from the lungs of infected hosts. These eggs were relatively free from extraneous material and were easily cleaned in several changes of water. If they were not to be used at the time of collection, they were placed in a refrigerator at 7° C. which temperature was sufficient to keep them from developing. They were incubated at 27° C. which appeared to be the optimum temperature. At this temperature, the immature miracidia are clearly distinguishable within two weeks and they are usually fully developed and active in three weeks, being capable of hatching at this time. Despite the thickness and coloration of the shell, mature miracidia are easily observed within the egg. A vitelline membrane surrounds the miracidium. One to several large, spherical, oily globules as well as granules of the remaining yolk are usually included within it.

With a few exceptions, eggs containing active miracidia remain for long periods of time in the incubator or at room temperature without hatching. Eggs left in an incubator for as long as two months or at room temperature for over 4 months after the miracidia had matured did not hatch. Garrison and Leynes (1909) reported a

similar retention of the miracidia for as long as 160 days. It was my experience that hatching was readily brought about by a sudden change in temperature, as by the addition of cold water. The most useful method of causing them to hatch was to place the dishes in the refrigerator at a temperature of 7° C. Eggs with mature miracidia left at this temperature for 5 to 10 minutes and then brought out into room temperature began to hatch in a few minutes. Eggs containing miracidia were often left in the refrigerator for 4 or 5 months or even longer without suffering injury. There was little evidence that any had hatched during this period.

Longevity. The longevity of eggs and miracidia is presented in two experiments upon eggs collected from the lungs of a pig October 18, 1930. In the first experiment, the eggs remained in the refrigerator at 7° C. until January 11, 1931, when they were placed in an incubator at 27° C. for about one month and were then returned to the refrigerator until February 24, 1932, when they were removed. A considerable number of the eggs hatched. In the second experiment, the remainder of the eggs were kept in the refrigerator until May 15, 1932. They were then incubated until June 17, 1932, when 8 per cent of the eggs contained living miracidia some of which hatched. Not all eggs hatch at the same time. This was convenient when the miracidia were being studied, for as soon as they began to appear, the dish containing the eggs was returned to the refrigerator and brought out only at short intervals. Thus, one collection of eggs provided a supply of miracidia for several weeks.

Conditions affecting development. A series of experiments in which the eggs were subjected to drying indicates that even a short drying is fatal, for no eggs developed when they were placed in water. That eggs will develop in a small amount of moisture was demonstrated when a large number of eggs were placed upon the top of some moist soil in a small dish which was put in an incubator. For several weeks a drop of water was occasionally added to keep the soil damp. Then water was withheld and the residual moisture permitted to evaporate until the soil had drawn away from the sides of the dish and had become hard and compact. Upon examination, over half (51 per cent) of the eggs still contained living miracidia most of which hatched when cold water was added. Almost every egg in a dish with water used as a control contained miracidia. As will be pointed out later, this ability of the egg to develop on damp soil is of importance in connection with the infection of the snail host. No experiments were made to test the resistance of eggs to freezing, but Garrison and

Leynes (1909) found that none were injured when frozen for 5 to 6 minutes but that an increasing proportion of eggs succumbed when the period of freezing was increased to 15, 20 and 25 minutes. No eggs developed after being frozen for 30 minutes.

Hatching. The act of hatching was observed on a number of occasions. Previous to the moment when this takes place, the only sign of life manifested by the miracidium is an occasional contraction of the body. Hatching can be foretold by a sudden increase in activity of the miracidium. It may at first lunge at the operculum but it soon flattens the anterior end against this structure, the surface of which it covers entirely and appears to anchor itself, though actually it is probably maintaining a steady forward movement. Then a series of violent contractions and extensions of the posterior portion of the body occurs until the operculum suddenly flies back as on a hinge. Water passes through the vitelline membrane by osmosis causing it to swell up and extend through the opening until it may become larger than the egg (plate I, fig. 14), forming a large, transparent sac into which the miracidium finally forces itself. It swims rapidly about until it succeeds in rupturing the membrane when it escapes and swims away. The vitelline membrane collapses and contracts, usually remaining as a small protrusion from the opercular aperture. Occasionally there is no protrusion of the vitelline membrane, the miracidium escaping directly from the egg as a result of the premature rupture of the membrane.

Miracidium.

Information on the morphology of the miracidium is meager despite the fact that considerable work has been done on the life history of *Paragonimus* in Japan and adjacent regions. Nakagawa (1917: 300) published a brief description of its general appearance and internal anatomy which consisted of "an alimentary tract and ganglion, two flame cells, and numerous embryonic cells."

Measurements. In the present study, measurements were made of 10 miracidia killed with heat and of the same number killed with cold 10 per cent formalin. All specimens were measured while confined in a minute amount of liquid free from pressure. Those killed with heat measured, 0.058–0.078 mm. by 0.036–0.049 mm. (av., 0.067 by 0.040 mm.); those with formalin, 0.060–0.102 mm. by 0.031–0.048 mm. (av., 0.081 by 0.041 mm.).

Morphology. The morphology of the miracidium of the lung fluke is rather simple. The body is normally pyriform in shape with

an anterior papilla and two lateral processes (plate I, fig. 15). While the whole surface at first appears to be ciliated, the cilia are located only on the 16 flattened epidermal cells which cover most of the body. The anterior papilla, lateral processes and the spaces between the epidermal cells are nonciliated. The epidermal cells are in 3 transverse rows, 6 in the first row, 6 (rarely 7) in the second, 3 in the third, with a single terminal cell at the posterior end of the body. The cells of the first row are triangular with their apices at the base of the anterior papilla, while their bases each with a conspicuous central indentation, are at the level of the lateral processes. The cells of the second row are rectangular, about one and one-half to two times as long and about the same breadth as those of the first row. The cells of the third row are about as long as those of the first and are broader than long. They may be rather cuneiform with bluntly pointed posterior ends. As a consequence, the terminal cell is more or less triangular. The ciliated epidermal cells are separated by narrow nonciliated areas which are the exposed portions of the subepithelial layer upon which the epidermal cells rest.

Cilia at the very apices of the first row of epithelial cells are short, being approximately 0.007 mm. long. They increase rapidly in length to about 0.013 mm. Cilia at the anterior ends of the second row of cells approximate this measurement, while the remainder of the cilia to the posterior end of the miracidium measure approximately 0.011 mm.

The only evidence of a nervous system is represented by a large, conspicuous ganglionic mass (plate I, fig. 16), located just below the level of the lateral processes. It may appear somewhat bilobed and may possess lateral extensions toward the lateral processes.

The remainder of the body cavity lateral and posterior to the brain is filled with germ cells of various sizes which stain particularly well in neutral red or Nile blue sulphate. Each contains a large nucleus and one or two conspicuous nucleoli. Many of the larger cells have fibrous extensions which appear to anchor them to the body wall.

The excretory system is represented by a pair of large flame cells located laterally just in front of the posterior margin of the second row of epithelial cells. The ducts are independent, extensive and convoluted, proceeding anteriorly from the flame cells for a short distance, then posteriorly to the junction of the third row of epidermal cells and the terminal cell where they loop back anteriorly to terminate laterally in a small bladder located between the second and third rows of epidermal cells.

Neither a gut nor penetration glands could be identified.

Longevity. At a temperature of about 25° C., miracidia usually do not live longer than 24 hours. Their greatest activity ceases long before this time. Miracidia at 7° C. may remain active for 3 or more days. Dying miracidia swell up, become transparent and move about more and more slowly.

Penetration into snail. Miracidia were observed to penetrate snails on but two occasions. In both cases, the miracidia penetrated the head and neck regions. Many were seen to enter the mantle cavities of other snails under observation. Nakagawa (1917: 302) and Kobayashi (1921: 10) observed that the ciliated cells were cast off in the act of penetration, but this was not seen by me. Snails exposed to large numbers of miracidia yielded as many as 5 large sporocysts containing greatly developed germ balls or first generation rediae showing that more than one miracidium may penetrate a snail and develop.

Old snails could not be infected experimentally, but an infection of almost 100 per cent was obtained in young snails of approximately one millimeter in length. It was necessary to obtain these in their natural habitat for the method of rearing them in the laboratory is not known. However, controls were maintained. After two weeks in the laboratory when sporocysts were recovered from the experimentally infected snails, 25 of the control snails were examined and no trace of a trematode parasite was found. One week later, 25 more controls were examined and were also negative. This evidence coupled with an almost 100 per cent infection in experimentally infected snails gives double assurance that the infections encountered in exposed snails were the result of experiment. Furthermore, when cercarial production was reached, only cercariae of *Paragonimus* were recovered. In adult snails collected in the natural habitat, the percentage of infection with *Paragonimus* is about one per cent.

The fact that *P. lapidaria* is an amphibious rather than an aquatic snail does not decrease the possibility of its becoming infected in its natural habitat. It can become infected while situated away from the water as well as in the rather brief periods when it is submerged. As already pointed out under the discussion of the egg, the presence of even a small amount of moisture is all that is necessary to promote development of the eggs. Thus, they may develop on the damp soil of the stream bank. Miracidia, when hatched, require very little water in which to move about, a mere film sufficing. A fairly heavy dew or rain or even the film of moisture which may seep from the

damp soil of stream banks in damp weather would make the snails accessible to miracidia.

Kobayashi (1921) is the only worker in the Orient who definitely succeeded in infecting snails. He also had to use snails from their natural habitat. Apparently the only studies he made were on sections of infected snails; consequently his information is meager. He reported finding sporocysts and first and second generation rediae but did not succeed in carrying the development to the cercarial stage. Therefore, his evidence is far from conclusive.

Sporocyst.

Location and morphology. Sporocysts occur free in the lymphatic system in practically every part of the snail; a few were found in the head, foot and liver but the majority were along the intestine, esophagus and stomach. This was determined by dissection and also by studying sections of snails exposed to miracidia. Sporocysts are somewhat ellipsoidal when young but become elongated as they develop and assume various shapes (plate Vb, figs. 92-96). Some are considerably distorted by the presence of first generation rediae. Several spent sporocysts found in snails which also had free first and second generation rediae were somewhat spindle-shaped (plate Vb, figs. 97 and 98). Fifteen mounted sporocysts measured 0.305-0.571 mm. by 0.086-0.133 mm. (av., 0.444 by 0.115 mm.).

Freshly removed sporocysts exhibited little activity. There usually is a more active attenuated end which is considered to be the anterior end. Most of the sporocysts were preserved immediately after removal from the snails and were stained and mounted.

The sporocyst is a sac-like body containing the developing first generation rediae (plate II, figs. 25-27), which seldom number more than 12 per individual. Many large cells with conspicuous nuclei and nucleoli as well as smaller, less conspicuous cells are present in the body wall. Groups of cells usually represented only by their nuclei and nucleoli are present at one or both ends of many individuals (plate II, fig. 27). These groups are of various shapes.

Longevity. The longevity of the sporocyst within the snail was not definitely determined, but a spent individual was recovered as late as 78 days after infection in a snail containing cercariae while one was found in another snail after 105 days where rediae only were present.

First generation redia.

Morphology of young rediae. Young first generation rediae were recovered from experimentally infected snails as early as 29 days after infection. Individual snails contained from 8 to 66 rediae, an average of 19 being recovered from 23 snails. Rediae were located in the lymph space adjacent to the intestine and stomach and in the liver. Twenty-five young specimens with a few germ balls measured 0.143–0.286 mm. by 0.076–0.143 mm. (av., 0.194 by 0.103 mm.). The mouth is terminal. The pharynx is conspicuously large, the smallest measuring 0.049 by 0.058 mm. and the largest 0.093 by 0.089 mm. with an average of 0.071 by 0.075 mm. The gut, rarely larger than the pharynx, measured approximately 0.057 by 0.060 mm.

Young rediae are typically short and truncate with an invaginated posterior end and prominent collar subtending the pharynx (plate II, figs. 35 and 36). There are no appendages. The cuticula is rugose because of the strongly developed longitudinal muscles. At times, the rugae when stained are so distinct on the collar as to suggest spines (plate II, fig. 36), while they may become so pronounced over the entire body in many old rediae as to practically obscure the internal structure in living specimens. The germ balls are clustered in a central mass.

Morphology of mature rediae. First generation rediae containing fully developed second generation rediae (plate II, figs. 28 and 29) were recovered in one lot of snails 63 days after infection. In another series, 70 days elapsed before this stage was found. Most mature first generation rediae resemble the young forms but some do not possess the prominent collar and may have somewhat rounded posterior ends. The birth pore is adjacent to the pharynx. Fifteen mature first generation rediae measured 0.238–0.409 mm. by 0.105–0.152 mm. (av., 0.293 by 0.133 mm.). The smallest pharynx was 0.067 by 0.080 mm. and the largest 0.084 by 0.098 mm. with an average of 0.076 by 0.084 mm. The gut ranged in size from 0.033 by 0.049 mm. to 0.058 by 0.062 mm. In some individuals, the gut is filled with amber-colored food material.

A first generation redia may contain about a dozen developing second generation rediae in all stages, but no more than 7 were ever found fully developed at one time; the usual number is 3 to 4.

Second generation redia.

Second generation rediae and cercariae from naturally infected snails have been described by Ameel (1931b, 1932b). They were

located in the lymphatic system in the liver and in that part of the system adjacent to the intestine and stomach. Rediae ranging in numbers from 9 to 62 per snail were recovered from 15 naturally infected snails. The average number was 22.

Morphology. The shape of the second generation rediae recovered from these snails is that of an elongated ellipsoid (plate II, fig. 41). There are no posterior or lateral appendages and no collar. The cuticula is finely rugose over most of the body. The mouth is terminal and opens immediately into a more or less spherical, muscular pharynx. The esophagus is short and thin walled, leading into a spherical or oval gut which is often smaller than the pharynx. The gut in old individuals is conspicuous on account of its content of amber-colored particles derived from the tissues of the snail. The gut wall consists of a single layer of large, flattened, clear cells with vesicular nuclei (plate II, fig. 38). The entire digestive tract is confined to the anterior third or fourth of the body. Some rediae contained as many as 20 to 30 fully developed cercariae. The birth pore is adjacent to the pharynx. Twenty mature second generation rediae killed in Gilson's solution and preserved as permanent mounts measured 0.332–0.819 mm. by 0.105–0.171 mm. (av., 0.559 by 0.139 mm.). The smallest pharynx measured 0.036 by 0.040 mm., the largest 0.056 by 0.058 mm. (av., 0.044 by 0.047 mm.). The smallest gut measured 0.039 by 0.022 mm., the largest 0.089 by 0.071 mm.

A small number of young rediae found in company with mature first generation rediae 70 days after experimental infection were identified as young second generation rediae because of the relatively small size of their pharynges. Their bodies were truncate, had rather pronounced collars and invaginated posterior ends. Numerous individuals of this same type were found in snails with older infections (plate II, figs. 30 and 31).

The two generations of rediae can be definitely distinguished by their structure and by their contents. The rediae of the first generation are relatively smaller than those of the second generation. The truncate form and prominent collars of the first generation rediae which are manifest even in most of the mature individuals serve to set them apart from the mature second generation rediae which have gradually sloping anterior ends and rounded posterior extremities. The large size of the pharynx of the first generation rediae is an especially good criterion in distinguishing them from the second generation rediae with their decidedly smaller pharynges. Mature first generation rediae usually contain about 3 to 4 fully developed second

generation rediae, rarely as many as 7, and a few germ balls, while mature second generation rediae may contain as many as 20 to 30 fully developed cercariae. Thomas (1883: 124) pointed out similar differences between the two generations of rediae with respect to size, pharynges, and content in his paper on the life history of the liver fluke, *Fasciola hepatica*. He also reported finding both rediae and cercariae in a first generation redia. No individuals of this type were observed in the present study.

Second generation rediae described by Nakagawa (1919), Ando (1919) and Kobayashi (1921) differ from those described above in the possession of large guts which extend either half the length of the body or almost to the posterior end. Nakagawa's and Kobayashi's drawings are reproduced (plate II, figs. 39 and 40). Since Ando's photographs of several individuals possessing unusually large guts could not be reproduced, the outlines have been traced and reproduced for purposes of comparison (plate II, fig. 37).

Cercaria.

Japanese investigators working in Japan, Korea and Formosa between 1916 and 1919 reported their discovery of the cercaria of the lung fluke. Nakagawa (1917) described a long-tailed cercaria produced in sporocysts which he found in a melaniid snail in Formosa and believed to be *Paragonimus*. He later discovered the proper cercaria (1919), and pointed out his earlier error. Iturbe and González (1919) working in Venezuela found a cercaria similar to that reported by Nakagawa (1917) and were of the impression that they had discovered the life cycle of the lung fluke in South America. This surmise, supported by inaccurate experimentation, is obviously erroneous, although it is certain that the metacercaria identified by them actually belongs to *Paragonimus*. Kobayashi (1921) presented the most complete available description of the cercaria of *Paragonimus* from Asia.

Morphology. The following description of the cercaria found in North America is based almost entirely on a study of living specimens. *Intra vitam* stains were used on the living material. The cercaria (plate II, fig. 42) is of the microcercous type with an ellipsoidal body and a small spherical or cylindrical tail. An oral and ventral sucker are present. The entire cercaria, including the tail, is covered with spines which are longest and most conspicuous on the posterior portions of the body and tail. The spines in these regions are often observed in preserved and mounted specimens. A group of large spines

on the tip of the tail is particularly conspicuous for the remainder of its cuticula is provided with very small spines. Unicellular, goblet-shaped, subcuticular glands are present over the whole body but are especially numerous at the anterior end. The acetabulum is oval, the greatest diameter being transverse, and is situated slightly posterior to the middle of the body. The spherical oral sucker is terminal.

There are 14 penetration glands with vesicular nuclei which can be differentiated into two types on the basis of their affinities for neutral red. On each side of the body lateral to the acetabulum, there is a lateral group of 4 large cells with a great affinity for neutral red and in the median area is a mass of 6 cells, usually smaller and poorly staining, situated just anterior to the acetabulum. Four large ducts leave each group of lateral cells, extend along the edge of the cercaria, penetrate the oral sucker, and open on each side of a large, simple stylet which is located in the anterodorsal portion of the sucker. Four ducts of about the same size as the lateral ones arise from the central cells, extend forward in the mid-region of the cercaria in two groups of two each and penetrate the oral sucker, opening two on each side of the stylet just posterior to the openings of the lateral ducts. In a preliminary paper (Ameel 1932b), three pairs of median ducts were reported but further study has satisfied me that there are but two pairs. Existence of ducts for the odd pair of median glands could not be ascertained by any technique applied.

A long prepharynx is present and a pharynx is located about midway between the oral sucker and the acetabulum. The remainder of the digestive tract could not be traced. The ganglionic mass is represented by a butterfly-shaped structure situated between the oral sucker and the pharynx, extending to the ducts of the lateral penetration glands. The genital primordium is present as a mass of cells posterior to the acetabulum. This mass of cells stains conspicuously in fixed specimens as well as in the living ones stained intravitaly. A median, thick-walled excretory bladder about as broad as the acetabulum occupies the region between the latter and the posterior end of the body. A collecting tubule arises at the anterolateral corner of each side of the bladder and extends to the edge of the cercaria where it receives an anterior and a posterior branch. From this juncture anteriorly to the penetration glands and for a short distance posteriorly, the excretory tubules form a conspicuous mass but they could not be traced farther. Thirty-one flame cells (plate I, figs. 1 and 2) were counted on each side of the cercaria of which over half

were located posterior to the acetabulum. No definite pattern could be determined. The measurements of 10 cercariae fixed in Gilson's solution and preserved as permanent mounts are as follows: length, 0.171–0.244 mm. (av., 0.196 mm.); width, 0.056–0.089 mm. (av., 0.079 mm.); tail, 0.013–0.018 mm. by 0.011–0.013 mm. (av., 0.015 by 0.012 mm.); oral sucker, 0.040 by 0.040 to 0.044 by 0.044 mm. (av., 0.042 by 0.042 mm.); stylet, 0.033 by 0.006 mm. to 0.038 by 0.009 mm. (av., 0.034 by 0.007 mm.); pharynx, approximately 0.018 by 0.022 mm.; acetabulum, 0.029 by 0.029 to 0.033 by 0.033 mm. (av., 0.029 by 0.034 mm.).

Development. The length of time necessary for cercariae to develop in experimentally infected snails appears to vary with the individual host. This is also true for the other generations in the snail. Two groups of experimentally infected snails kept at the same temperature gave the following varied results in respect to the degree of development of the parasites. Active, full-sized cercariae and well developed first generation rediae were recovered from two experimentally infected snails in one collection 93 days after infection, although several other snails of the same collection examined at the same time contained only partially developed first generation rediae. Six well developed cercariae and a number of second generation rediae were recovered from a single snail in another collection 78 days after infection. This is the shortest period after infection that cercariae were found. At the same time, 7 snails from the same lot contained immature first generation rediae; two others contained both mature and immature first generation rediae while another snail contained first generation rediae in various stages of development and others which appeared to be young second generation rediae. Thirty days later cercariae were recovered from but two out of 10 snails from this same collection while only rediae were found in the others.

Morphology of immature cercariae. All cercariae recovered from two of the above snails and about 9 per cent of those from naturally infected ones possessed, in addition to penetration glands, two kinds of gland-like cells which appeared to be devoid of ducts (plate II, fig. 43). These cells were differentiated on the basis of size, some being large and some small. With one exception, the large cells with vesicular nuclei and fine cytoplasm which stained brilliantly with neutral red were situated in the area posterior to the acetabulum and numbered from 2 to 6, with 4 as the usual number. In some fixed and stained cercariae from one snail, it is apparent that there are as many as 12 to 14 of these large cells. They are arranged in pairs and

the group extends from the posterior end of the body almost to the oral sucker. Many of the cells are as large as 0.024 by 0.027 mm. The small gland-like cells with vesicular nuclei and a coarse, poorly staining cytoplasm were located along the margins of the cercaria and occasionally in the mid-region between the suckers. These gland-like cells of both large and small types were never observed in normally emerged cercariae and may represent a stage in the early development of the cercaria.

Kobayashi described and figured a median series of large gland-like cells in addition to the penetration glands (plate II, fig. 44). His description agrees in every respect with the writer's observations on the large gland-like cells found in the developing cercariae in the single snail mentioned above. Correspondence with Kobayashi elicited the information that he had used cercariae from crushed snails exclusively and had not studied them alive with the aid of *intra vitam* stains but had studied them only as fixed and stained specimens.

Nakagawa's description of the cercaria is very meager but his figure (plate II, fig. 45) is suggestive of the normally shed cercariae studied by me. Faust (1922) described a microcercous cercaria (*Cercaria abbrevicauda*) from a melaniid snail from Kiukiang, Kiangsi, China. He postulated that this probably was the cercaria of *Paragonimus* since the cercaria bore some resemblance to that described by Kobayashi and came from a melaniid snail collected in a region where the presence of the lung fluke had been recorded. Later (1929) in his text book he presented this cercaria as the actual cercaria of *Paragonimus*, though in the text he stated that there are no authentic endemic records of human paragonimiasis in China. As far as the writer can ascertain, there are no reports on the occurrence of *Paragonimus* in any other type of host in China than man (see Khaw, 1930). The anatomy of this cercaria, if accurately described practically precludes the possibility that Faust was dealing with any known species of *Paragonimus*, but he may have had some other member of the family *Troglotreumatidae*.

Activity. The cercariae emerge from snails in the laboratory in the greatest numbers in the late afternoon or at night. This coincides very well with the habits of the crayfishes which are most active at night. The cercariae are positively phototropic, congregating in the most highly illuminated portion of the dish. Their progression is leech-like and even though they may be floating through the water, they execute the same movements. They cannot swim, but, when the

water is sufficiently agitated, they may release their hold and float along in the current. At times, they secrete considerable quantities of mucus in which they become entangled in squirming masses. At other times, individuals were observed anchored to fragments of debris by a strand of mucus which they utilized as a sort of tether, floating free and executing their creeping movements. Cercariae at room temperature survive from one to two days while at 7° C. many live a day or two longer.

In their natural habitat, the snails probably give off their cercarial infections to a film of moisture provided by dew, rain or seepage just as readily as they do when submerged in the water. Since at night humidity is usually so high as to stop evaporation, nearly or even completely, a film of water may appear during the night where it is absent during most of the daylight hours.

Penetration into next host. When cercariae were placed in a dish with crayfishes, they were observed to penetrate the thin chitin on the under side of the tail at the junction of the segments. Probably they also attack the crayfish at the segments of the appendages and other vulnerable regions. No doubt, they can penetrate the soft exoskeleton at any point for a short time after ecdysis.

Metacercaria.

Nakagawa (1916) and Ando (1915) first reported on the metacercaria of *Paragonimus*. Nakagawa discovered the metacercariae infesting three species of fresh water crabs in Formosa. He found a small encysted metacercaria localized in the liver and a much larger form encysted most numerous in the gills. He believed that the smaller encysted worms were merely immature forms of the larger. However, Yokogawa (1917) later discovered that the small metacercariae developed into a different species of fluke in the bile ducts of experimentally fed mice. The larger form was found to be *Paragonimus*. Kobayashi (1918) presented a study of the gross and histological morphology of the metacercaria and while most of the present work is merely confirmatory, several hitherto unreported structures are described.

Location in host. According to Kobayashi (1918: 103), the cysts in the crayfish (*Cambaroides*) "are usually found in the muscles or the viscera of the cephalothorax, especially in the region of the endophragmatic skeleton. The gills, the ambulatory legs and the muscles of the abdomen are also infested, though small in number. . . . In the crabs the gills and the muscles of the proximal part of the am-

bulatory legs are usually infested.''' Other Japanese workers agree on these findings. In the North American crayfishes of the genus *Cambarus*, the cysts are always localized in the cardiac region, especially upon the heart but sometimes either within blood vessels leaving the anterior or posterior part of the heart or attached to the wall of the pericardial cavity. When located upon the heart, the cysts are either on the internal or external walls adjacent to the ostia. Thus, the cysts are arranged in a band across the heart (plate VI, fig. 100). The external cysts are usually most numerous on the dorsal and lateral surfaces. Cysts are not embedded in the tissues but are attached to it by an enveloping membrane. Kobayashi reported finding as many as 1016 cysts in a single crayfish though the usual number ranged from one to several hundred. The majority of *C. propinquus* collected from a small stream near Ann Arbor contained 20 to 30 cysts though 40 was not uncommon, while one individual 72 mm. long contained 147. Even in such a heavy infection, all the cysts were confined to the pericardial cavity, a careful examination failing to reveal any in other parts of the body.

Morphology of encysted metacercaria. Mature cysts (plate II, fig. 21) are oval, measuring 0.381–0.457 mm. by 0.381–0.447 mm. (av., 0.420 by 0.406 mm.), when free from pressure. The cyst wall is thick, being about 0.056–0.067 mm. in section. It is very transparent and permeable; neutral red passes through readily and stains the enclosed worm without staining the wall itself. A thin membrane formed by the metacercaria and usually closely adherent to the cyst wall encloses the cyst proper and anchors it to the host tissue. Kobayashi (1918: 105) claimed that this membrane is formed by the host but this cannot be since it is non-cellular.

Metacercariae within the cyst are contracted, seldom doubled on themselves. The most conspicuous feature of mature, living encysted metacercariae is the large excretory bladder filled with highly refractive excretory granules extending practically the full length of the cyst. This area appears pure white in reflected light and black in transmitted light.

Large, convoluted intestinal ceca fill up a large part of the larva not occupied by the excretory bladder. Metacercariae from a single crayfish examined shortly after it was collected contained reddish globules. These globules were present in almost every metacercaria in a collection of crayfishes kept in the laboratory for several months. So many globules were present in some instances as to give the metacercariae a pinkish cast recognizable to the naked eye. They

were of various sizes, generally spherical to oblong, occurring singly or in groups, and were most numerous in the posterior portion of the worm. The tissues of the crayfish overlying the pericardial cavity often contain numerous red pigmented globules which may be the source of the reddish pigment observed in the metacercariae with which it appears to be identical. This reddish pigment in the metacercariae has been noted by several Japanese investigators. Occasionally, cysts, probably old, are found surrounded by a reddish-brown encrustation.

Survival of metacercariae outside the host. Encysted metacercariae removed from the crayfish are capable of surviving under different physical conditions for various lengths of time. Two of a collection of 31 metacercariae in crayfish viscera kept at 12°–21° C. remained alive after 5 days. Four of 6 encysted metacercariae at the same temperature but free in water survived for 10 days. Two of 6 metacercariae in water survived for the same length of time at 18°–24° C. Twenty-nine metacercariae were kept in crayfish viscera at this temperature for three days. They were vesicular and moved but little. They were fed to a cat and 10 worms were recovered when it was examined. Another cat was fed 30 metacercariae that had been in crayfish viscera at 7° C. for 19 days. Eight adult worms were recovered.

The effects of a 10 per cent salt solution and of 35, 12, 6 and 3 per cent solutions of alcohol were observed upon encysted metacercariae freed from host tissues. The larvae in 10 per cent salt solution and those in 35 per cent alcohol were killed almost immediately, but they remained active in 12 per cent alcohol for 10 minutes, in 6 per cent alcohol for 1¾ hours, and in 3 per cent alcohol for 2¾ hours. Various Japanese workers have performed similar experiments with varying results.

In Fukien, Kwantung and possibly Kiangsi provinces in China, crabs and crayfishes are cut into small pieces and are placed raw in Chinese soy sauce, vinegar, salt and sugar where they are permitted to remain for about three hours before being served. They are also prepared in like manner in native wine. On two occasions, a Chinese student prepared a small dish of crayfishes containing a large number of metacercariae in this sauce, following the same recipe she had used in China. After three hours, the cysts had become opaque and appeared dead. They were fed to cats which were negative upon later examination. The fact that these metacercariae were killed does not remove the danger of eating crayfishes prepared in this

manner for, no doubt, within a certain period they are infective, and some larvae which do not come into good contact with the sauce may remain alive. When the metacercariae occur within the tissues, as in the Asiatic crabs and crayfishes, they are probably killed by the sauce only after a prolonged period of immersion. Haste or carelessness on the part of a cook may expose the diners to infection. As pointed out by Khaw (1930) who cited several cases, Chinese, while residing in foreign countries where the lung fluke is prevalent, continue to prepare dishes of raw crabs and crayfishes using their native recipes, with regrettable results to themselves.

Abend (1910) reported a case of paragonimiasis which was undoubtedly contracted in this country. The patient, a German who had resided in the United States for 20 years, had worked with a railroad construction crew in Texas, Colorado and St. Louis and, probably, also in contiguous territory. His food had been prepared largely by Chinese cooks. He appeared before Abend, upon his return to Germany, suffering from hemoptysis. Abend, upon examining some of the blood-stained sputum, discovered that it contained the eggs of a trematode which he identified as those of *Paragonimus*. To explain this infection, it must be assumed that the man had eaten infected crayfishes procured in a region where he had worked and probably prepared by a Chinese cook using his native recipes.

Excystment of worms for my study was at first produced by the successive use of artificial gastric and pancreatic juice at 37° C. but, when for some unknown reason, this method failed, those that emerged when placed in tap water were the only ones available. The larvae escape from the cyst through a slit.

Some of the worms were studied alive with the aid of neutral red while others were killed with heat, fixed in Gilson's solution and made into permanent mounts.

Morphology of excysted metacercariae. Living metacercariae are capable of considerable extension, appearing thin and leaf-like when extended. The excretory bladder (plate I, fig. 23) is a conspicuous structure due to its content of excretory granules.

Mounted specimens (plate I, fig. 22) are ellipsoidal in shape. The body from the anterior edge of the acetabulum back is rather uniformly broad while the region anterior to this tapers quite abruptly to the oral sucker. Twelve specimens measured 0.524–0.866 mm. by 0.209–0.295 mm. (av., 0.623 by 0.239 mm.).

The cuticle is thick and beset over the whole body with a dense array of small, needle-like spines. Both the oral sucker and the

acetabulum are well developed and muscular. The oral sucker is smaller, ranging in size from 0.062 by 0.067 mm. to 0.084 by 0.084 mm. The anterior portion of the cercarial stylet remains in the anterodorsal part of the sucker and measures 0.009–0.022 mm. (av., 0.013 mm.) by 0.004 mm. The acetabulum is slightly anterior to the middle of the body and ranges in size from 0.067 by 0.111 mm. to 0.133 by 0.127 mm.

The long prepharynx of the cercaria is absent, the pharynx being closely appressed to the oral sucker. The pharynx may be spherical or wider than long. Measurements of individual pharynges ranged from 0.033 by 0.036 mm. to 0.038–0.038 mm. The esophagus is relatively short. The intestinal ceca are quite narrow at their bifurcation but soon become broad and voluminous.

There is a group of about 14 small, well-defined gland cells with large vesicular nuclei lying on each side of the bifurcation of the ceca. These cells have been overlooked by previous investigators. Their ducts, which are both median and lateral, extend anteriorly to the oral sucker where they are obscured by the heavy musculature of that organ. Their function is problematical. None were found in metacercariae recovered from white rats later than three days after ingestion. Since experiments which will be discussed later prove that the undeveloped worms lying free in the body cavities of white rats are infective for at least 185 days, the glands are evidently not required by the young worms in their migrations in the final host, but may be used by the metacercariae in escaping from the cysts. For this reason, it is proposed provisionally to call these structures excystation glands.

Another heretofore unreported group of structures was observed in living worms. These are about 20 to 30 or more clusters of spherical vacuolate bodies situated adjacent to the outer edge of the ceca (plate I, fig. 23) from the level of the acetabulum to the posterior end of the body. They are not affected by neutral red. No suggestion is offered for their function.

The ganglionic mass with anterior and posterior rami lies just posterior to the pharynx.

The excretory bladder fills up the entire central portion of the worm between the ceca from their bifurcation to the posterior end of the body. It is a straight, sac-like structure with a rounded anterior end and a posterior extremity tapering to the excretory pore. Occasionally in living specimens with partially filled bladders and always in sectioned material, villi of various lengths may be observed

projecting into the lumen of the bladder (plate I, fig. 18). The extensive excretory system of vessels emptying into the bladder (plate I, fig. 23) is easily observed in living material. A collecting canal leaves each side of the bladder just posterior to the acetabulum and extends slightly anteriad to the outer edge of the intestinal cecum where it gives rise to an anterior and a posterior branch, each with numerous lateral branches.

The primordia of the entire reproductive system are recognizable in some well stained specimens. The testes are represented by small masses adjacent to the excretory bladder about midway between the acetabulum and posterior end of the body. The primordia of the ovary, uterus, oötype and Mehlis' gland lie just posterior to the acetabulum.

Development. Development of a metacercaria from the time of encystment to the time it becomes infective is largely dependent upon the temperature. Development proceeded as follows in several collections of crayfishes infected during the month of September, 1932, and kept in an aquarium room at a temperature of approximately 20° C. Cercariae appeared in the heart 12 hours after the crayfishes were exposed to infection but none were found encysted until about 12 hours later. They were then enveloped in a thin membrane which was attached to the host tissue. The cercarial penetration glands and ducts were still intact but three days later the median ducts had disappeared while the remaining ducts and glands still retained their identity. A thin inner cyst membrane was detected the fifth day after infection. Only a few small fragments of the penetration glands were observed on the eleventh day.

In 22 days, certain changes were noted in the metacercariae. The excretory bladder was evident and broad, extending almost all the way to the pharynx, but it contained few excretory granules. Even the villi were present. The pharynx was adjacent to the oral sucker, though in 16-day worms, it was still a short distance from the sucker. The intestinal ceca of which slight evidence was first found in 17-day metacercariae were distinct but narrow and delicately outlined. Average dimensions of 10 encysted metacercariae free from pressure were 0.285 by 0.220 mm. Three stylets measured 0.023, 0.035, 0.037 by 0.007 mm.

At 5 weeks, the organs as well as the metacercariae themselves were materially larger (plate I, fig. 20). The excretory bladders were loosely filled with excretory granules and appeared brownish-gray in color by transmitted light. Forty-six day metacercariae had

attained full size and, when fed to a cat, developed into adults. At this age, the excretory bladders were full and appeared black in transmitted light. The stylets were not fully reduced to the average size found in older metacercariae and the body was more opaque than normal. It took several weeks for them to become of average clearness after they had become full grown. Excystation glands were not identified with certainty until the metacercariae were 9 weeks old.

Method of acquiring infection. No doubt, all of the mammals serving as hosts of *Paragonimus* may become infected through the eating of the second intermediate hosts bearing metacercariae. A mink, when given crayfishes, ate them voraciously. A caged muskrat was induced to eat crayfishes when green food was withheld. Sufficiently starved cats readily ate crayfishes. Seton (1929) states that the bobcat eats crayfishes and that others of our carnivores such as the lynx, wolf, coyote, red fox and grizzly will even eat insects and other small forms of life. If they partake of this type of food, they might not hesitate to eat the crayfish. The diet of Asiatic carnivores may also include insects, crustaceans and similar food, but no records on this point have come to my attention.

It is also possible for a definitive host to become infected through eating another definitive host containing more or less undeveloped worms which have not entered the lungs. This has been demonstrated by several Japanese workers. A series of successful experiments involving a first and second definitive host were conducted by the writer with the following results which have been previously reported (Ameel 1931a, 1932b). Twelve 185-day old worms from the pleural cavities of several white rats were fed to a cat known to be free from *Paragonimus* and 6 adult worms were recovered 7 weeks later. A series of experiments involving worms of successively shorter periods of time from both the pleural and abdominal cavities were successful without exception. However, a feeding of 24 worms (2 to 3 mm. long) obtained from the lungs proved unsuccessful. It has not been possible to determine that these young worms remain in the body cavities of mink as they do in white rats; nevertheless, the above experiments are sufficient proof to indicate that relatively undeveloped worms which have not entered the lungs are infective if their host is eaten. This may explain the method by which mammals that rarely or never eat crayfishes may acquire an infection. Ameel (1931a) reported that trappers claimed to have witnessed the eating of mink by dogs, cats and foxes. He also reported the eating of mink by several laboratory cats. It should be added that the cats readily ate the food without being starved.

Another possible, but probably not important, mode of acquiring an infection is by swallowing free, viable cysts in the drinking water. This has been suggested by Yoshida (1916). Kobayashi (1921) also suggested the possibility but placed little credence in it. It has been determined that cysts remain alive in water for considerable lengths of time depending upon the temperature. Yoshida found that cysts could be kept alive in running water for at least 25 days. The writer, as already mentioned, infected a cat with cysts kept in crayfish viscera at 7° C. for 19 days. It is easy to surmise that, under natural conditions, cysts could be freed from decomposing crayfishes in situations accessible to mammalian hosts, yet it is doubtful if this method of infection is really significant. The number of such free cysts swallowed must be few.

Migration within final host. Nakagawa (1917) made a careful study of the course taken by the metacercariae after they were ingested by the final hosts. His findings indicate that metacercariae enter the abdominal cavity by boring through the small intestine in the region of the jejunum. They then make their way to the pleural cavity by penetrating the diaphragm at the ligament or in the muscular portion. The first process took 24 hours or more while the second took 77 hours or longer. Nakagawa found evidence that the worms penetrate the lungs three days after the metacercariae had been swallowed, but no pronounced lesions were found until about 14 days after infection.

The writer found 7 young worms in the abdominal cavity of a white rat 4 hours after infection. Seven still encysted worms were found in the stomach and one in the duodenum. They were found only in the abdominal cavity of another rat killed 8 hours after infection. Worms were found in the pleural cavity of a white rat first after 96 hours of infection and in the lungs 14 days after they had been ingested. They normally localize in the lungs but investigators have found them in almost every part of the body, especially in man. Numbers of worms fail to penetrate the lungs of white rats and some remain free in the body cavity for at least 259 days.

Adult.

Morphology. Since the anatomy of the adult has been well described by various investigators, the more recent studies being those of Kubo (1912) and of Ward and Hirsch (1915), it need not be re-described here.

Measurements. Ten completely developed, unflattened specimens

from the cat measured by me averaged 11.4 mm. in length, 6 mm. in width and 4.9 mm. in thickness. Seven specimens from the mink averaged 10.8 mm. in length, 6 mm. in width and 4.1 mm. in thickness. Some of the flukes collected from the muskrat were noticeably larger than those recovered from any other host. Four unflattened specimens averaged 13 mm. in length, 7 mm. in width and 6 mm. in thickness.

Time of sexual maturity. Worms from experimentally infected cats were found to be sexually mature $5\frac{1}{2}$ to 6 weeks after infection and eggs were first observed in the feces at this time. Six week old worms measure approximately 6 by 3 mm. and it is obvious that they undergo a considerable increase in size after attaining sexual maturity. The worms raised in white rats rarely exceeded 5 mm. in length. Their small size may be explained by their restricted habitat or attributed to the fact that the rat is not wholly suitable as a host, perhaps for physiological reasons.

Cysts in lungs. Usually there are two worms within each cyst in white rats, cats, dogs and pigs. Overcrowding in white rats and cats sometimes raises the number to more than two. In several instances, 4 worms per cyst were found in lightly infected muskrats and as high as 6 per cyst in mink. The cysts in the lungs of white rats, muskrats and mink as well as in the thinner portions of the lungs of cats (plate VI, fig. 101), dogs and pigs are prominent bluish-gray protuberances. Some cysts are so deeply imbedded in the lungs of the larger hosts that they are not evident on the surface. Their presence is indicated largely by the brownish appearance of the lung tissue produced by the great number of eggs that lodge there. The greatest number of cysts are located in the posterior lobes of the lungs. They are usually about the size of a hazel nut, though in white rats, they are much smaller.

Longevity of adult worms. There is very little reliable information on the length of life of the adult flukes but indications are that they may live for a considerable number of years. Cases of infection contracted by people inhabiting endemic areas do not furnish reliable information for there may be a recurrence of infection. However, Fehleisen and Cooper (1910) report the case of a Japanese, resident in California for 6 years, who was suffering from paragonimiasis which had been diagnosed as such previous to his departure from the Orient. The case of Abend (1910), already mentioned, is remarkable in that its history covers a period of 23 years. The patient who came to Abend's attention in a German hospital in 1896 claimed to have

suffered from the same symptoms for 10 years previously while residing in the United States. The length of this period may be discounted in part, due to the possible unreliability of the patient's memory and, also, to the possibility that he may have received infections at intervals prior to his departure from the United States. However, the last symptoms did not disappear until 1909. The writer infected a cat January 18, 1931, and was still recovering large numbers of eggs in its feces at the time it escaped, March 11, 1933.

Distribution.

A knowledge of the general distribution of *Paragonimus* may be secured from Khaw (1930).

North American records following that of Ward (1894a) are: Kellicott (1894) in a dog at Columbus, Ohio; Stiles and Hassall (1900) in hogs slaughtered at Cincinnati, Ohio; Abend (1910) in a German miller; Nickerson (1911) in a cat at Minneapolis, Minnesota; Hirsch (Hanson, 1911) in 3 cats at Wauwatosa, Wisconsin; Smith (1911) in a wild cat from South Carolina; Hall (1925) in a goat probably from Brooksville, Mississippi; Henry (1927) in 2 mink from Minnesota; Feldman and Essex (1929) in a cat at Rochester, Minnesota; Riley (Wallace, 1931) in a dog from Ithaca, New York, and a cat from Minneapolis, Minnesota; Wallace (1931) in mink and crayfishes in Minnesota; Ameel (1931a) in a cat near Ann Arbor, Michigan, and in mink and crayfishes from the lower peninsula, also in muskrats from Muskegon and Manistee, Michigan (Ameel, 1932a). Null (1910) incidentally mentioned the presence of *Paragonimus* in cats and dogs in the Oriental quarter in San Francisco. No reports regarding the presence of the lung fluke in California have been made since that date and it is suggested that the infected animals may have been brought from the Orient. Prof. Ned Dearborn of the Forestry Department, University of Michigan, in 1918 found *Paragonimus* in a mink from Wauzeke, Wisconsin, and in two mink raised in confinement at Campbellville, Kentucky (unpublished records). The specimens were identified by Ransom. Prof. George R. La Rue has in his collection specimens from a cat from Hillsdale, Michigan (unpublished record).

Method of determining distribution. The metacercaria of *Paragonimus* was found to be more useful than the adult in ascertaining the distribution of this parasite, for its host, the crayfish, is generally very numerous and presents no difficulties in collection such as are encountered in the securing of the mammalian hosts. It differs dis-

tinety from other metacercariae found in the cardiac region of the crayfish, and is easily identified either in the living or preserved state. Its localization usually in the heart and adjacent blood vessels permits an easy and rapid examination for its presence. Crayfishes were secured from a large part of the United States and from southern Ontario. The carcasses of fur-bearing animals from Michigan and northern Ohio were examined also for they were easily secured from this area.

The accompanying map (plate Va) indicates those places where infected crayfishes and mammals were found. The large number of records indicated in Michigan is due in part to a more thorough survey of this state. The Minnesota records are unpublished data contributed by Dr. F. G. Wallace. From the map and past records it is possible to ascertain the limits within which *Paragonimus* is known to exist. Though both crayfish and snail hosts appear to be fairly abundant in Kentucky and Tennessee, the writer failed to find *Paragonimus* in a large number of crayfishes from these states. Evidently the fluke may be present in these states as well as in many others from which, to date, there are no reports of its presence. The conditions are apparently ideal for maintenance of the life cycle in many regions and it is hoped that future investigations will add considerably to the existing knowledge of the distribution of *Paragonimus* on this continent.

Taxonomy.

Prior to the work of Ward and Hirsch (1915) only such characters as the size and structure of the egg and the size and shape of the body and internal structures of the adult were considered in distinguishing the species. These characters are extremely variable even in individuals of the same species and, therefore, are of little value.

Ward and Hirsch, basing the species differentiation on spine structure, believed that they had a constant criterion. They described the spines of *westermanii* as being lancet-shaped and sparsely scattered in more or less incomplete circular rows, the spines of *ringeri* as chisel-shaped and arranged in groups sometimes close enough in certain regions to form almost complete circular rows around the body, and the spines of *kellicotti* as chisel-shaped and heavy with serrated free extremities and always distributed singly, like those of *westermanii*. The result of this differentiation was to place the species geographically as follows: *P. kellicotti* in North America, *P. westermanii* in India, and *P. ringeri* in all other endemic regions in Asia. According to their report, the material upon which Ward and Hirsch

made their study consisted of several specimens from man in Japan, three co-types of *D. westermanii*, and a series of North American lung flukes. The authors realized the inadequacy of their material and pointed out the need for a more complete study on a larger supply of material.

Kobayashi (1918) examined specimens from Korea and Japan taken from naturally infected man, cats, dogs and pigs and from experimentally infected cats, dogs, rabbits and rats and found that the general type of spine in the adult was flat and chisel-shaped, like those of *kellicotti*. He expressed the opinion that Ward and Hirsch were dealing with individual variations and claimed that "the discrepancies found in the system of *Paragonimus* can only be effaced by further investigations on the development of American lung-flukes."

Vevers (1923) worked with material from the mongoose and leopard cat (*Felis bengalensis*) from India, tiger (*Felis tigris*) from the Malay States, a specimen of *P. kellicotti* from the United States, and type specimens of *P. ringeri* and *P. compactus* borrowed from Prof. R. T. Leiper. Besides emphasizing the utility of the spines in species differentiation, he stressed the size of the egg and adult as characters for differentiation. He found that the specimens of *P. compactus* from the mongoose had cuticular spines grouped like those of *ringeri* but that they were lanceolate in form, thus distinguishing this species from the latter.

In the present study, 75 worms of various ages were used from local naturally or experimentally infected hosts. In addition, the following specimens were secured: Three specimens from a dog in Formosa supplied by Dr. Sadamu Yokogawa of the Medical College of Formosa, 3 specimens from a dog and two from man in Korea supplied by Dr. Harujiro Kobayashi of the Korean General Hospital, two specimens from a tiger in the Malay States supplied by Prof. R. T. Leiper of the London School of Tropical Medicine, and one specimen from a man in Japan and one specimen from a tiger in Sumatra as well as 6 slides of sectioned material from the tiger from two sources supplied by Prof. E. Brumpt of the Université de Paris. Dr. Edwin Hirsch kindly furnished 3 slides of the mounted cuticula of *P. ringeri* and one of *P. kellicotti* that had been used by himself and Ward in their study of the spines.

The cuticula was removed from all parts of the body with a razor, freed from adhering tissues with the aid of dissecting needles, and mounted in euparal with the external surface up. Water glass proved to be of considerable value as a mounting medium if the mounts were

for immediate use but, since they soon deteriorated, this medium cannot be recommended for permanent mounts.

The spines of local material are normally arranged over the body in more or less circular rows. They extend from the inner to the outer surface of the cuticula, penetrating it, and are directed posteriorly at an acute angle. The suckers and a small area around them are free from spines. A large number of spines immediately surrounding the acetabulum are misshapen (plate III, figs. 63 and 64). Contrary to the description of Ward and Hirsch (1915), spines do not always occur singly but occasionally are in groups of two (plate III, figs. 59, 66, and 67) or three (plate III, fig. 58). When the spines are grouped in this manner, their usual relative shapes and their manner of association indicate that they probably originated by the splitting of a single spine. Normal spines are subject to considerable variation in size and shape, and differ in size on various parts of the body. They may be flat and chisel-like as formerly believed, but many are cylindrical (plate III, figs. 49, 53 and 61) or cone-shaped while the exposed portion, and often the base, is usually serrate. Metacercariae and slightly developed worms have needle-like spines (plate III, figs. 46 and 47) but as the worm develops, the bases of the spines increase in size (plate III, fig. 48) and the extremities become more blunt as the points are worn off (plate III, fig. 51). No glands associated with the production of spine material could be definitely identified with the ordinary technique employed, but it must be admitted that no special techniques were used for this part of the study. Adjacent spines maintain their individuality throughout development and do not fuse to form the broad spine of the adult as Vevers (1923: 12) claimed. However, a broad spine may split to form several as Kobayashi (1918: 112) has pointed out.

All of the specimens from Korea and Formosa were carefully examined and, without exception, the spines (plate IV, figs. 69-77) were of the same general type as those found in North American specimens. Spines from the specimens from Sumatra were also of this type (plate IV, figs. 87, 89 and 90). The specimens from the Malay States, possibly from the same collection Vevers worked upon, had spines of the *kellicotti* type (plate IV, figs. 84, 87 and 88) which verifies his identification. However, the spines in several areas of cuticula from one of these worms mounted in water glass and subjected to considerable pressure resembled the *ringeri* type (plate IV, fig. 85). No spines of this nature could be found in cuticula from adjacent areas of the same worm mounted after the ordinary method

in euparal, but they were present in the cuticula of this worm when mounted in euparal and subjected to pressure. The spines were deeply sutured and probably fractured under the unusual pressure. This possibility should always be considered when making a study of the spines. Herein lies the danger of dealing with a small amount of material.

The majority of the spines of the material sent by Hirsch, labeled *P. ringeri*, agreed fairly well with his description of that species (plate IV, figs. 78, 80, 81 and 83) but, nevertheless, a few spines were found that resembled those of *P. kellicotti* (plate IV, figs. 79 and 82).

There was no opportunity to examine the slides from which the spines of *P. westermanii* were described but several specimens of local material yielded scattered samples of spines resembling Ward and Hirsch's description of this type (plate III, fig. 57).

It is evident from the above discussion that the differentiation of the species of *Paragonimus* by means of spine characters is far from a settled question. Although the writer's evidence indicates the possibility that there is but one species, it is not based on sufficient material to justify a statement to that effect. Further study awaits some one with a large series of Asiatic material from each of the regions where the disputed species are endemic. However, the above evidence is sufficient to indicate that cuticular spines may be poor criteria for the differentiation of species.

It is believed that more satisfactory characters may be provided in the stages of the life history and these should be sought. This paper presents a thorough study of the life history stages for the North American form but a detailed comparison with the life history of the Asiatic form is impossible because no complete study of it has been made. At this time, the following analysis can be presented. There are no important points of difference in the metacercariae as studied up to the present. The cercaria described by Nakagawa (1919) though lacking detail, is suggestive of the naturally emerged cercariae found in North America. The similarity of Kobayashi's description and figure to cercariae secured by dissection of the snail by me has already been discussed. Indications are that a thorough study of the cercaria in Japan and in other parts of Asia must be made before any definite conclusion as to its identity can be reached. The only striking dissimilarity found between the Asiatic and the American worms is in the size of the gut of the second generation rediae. As mentioned earlier in the discussion of the second generation redia, whereas the gut in the North American form is usually but

little larger than the pharynx, three Japanese investigators commented upon the large size of the gut of the second generation rediae in their material. They described it as extending over half the length of the body or almost to the posterior end. For a comparison of the relative size of the gut in American and Asiatic rediae, see plate II, figs. 37 and 39-41.

If the taxonomy of *Paragonimus* species is to be based upon life history studies, it should be borne in mind that no satisfactory determination can be made until a detailed knowledge of the life history stages is available for many endemic areas in Asia and other parts of the world. It is altogether too much to assume *per se* that the lung flukes from all the endemic areas of the world outside North America present precisely the same morphological pictures throughout their life histories as is known for this fluke in Japan.

For the present, in the absence of conclusive evidence either to support or contradict the work of Ward and Hirsch, it is believed advisable to recognize the species considered valid by them, namely, *Paragonimus westermanii*, *P. ringeri* and *P. kellicotti*. Likewise, *P. compactus*, recognized as a distinct species by Vevers, should be retained until it can be restudied.

Summary.

1. All stages of the life history of the lung fluke of mammals in North America have been worked out in detail.
2. The usual mammalian host is the mink.
3. The second intermediate host is various species of crayfishes of the genus *Cambarus*.
4. The snail host is *Pomatiopsis lapidaria*.
5. All four generations in the snail have been reared in experimentally infected snails.
6. The sporocyst and first generation redia of *Paragonimus* have been described in detail for the first time.
7. The two redial generations have been distinguished on a structural basis.
8. Hitherto undescribed morphological structures are reported for the miracidium, first and second generation rediae, unemerged cercaria, emerged cercaria and metacercaria.
9. The knowledge concerning the distribution of the lung fluke of mammals in North America has been considerably enlarged.
10. Sufficient evidence has been presented to indicate that the cuticular spines of the adult may not be good criteria for species

differentiation. It has been suggested that characters more suitable for this purpose may be found in the life history stages but that, to be of value, it would be necessary to work out the life history in many endemic areas. The size of the gut in the second generation redia was pointed out as the one difference between the lung flukes of North America and Asia.

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EXPLANATION OF PLATES.

PLATE I.

All drawings made with the aid of a camera lucida.

FIGS. 1 and 2. Flame cell arrangement in two individual cercariae, living specimens.

FIGS. 3 and 4. Eggs recovered from feces showing the outlines of the yolk cells and ovum, living specimens. (All eggs drawn to the same scale.)

FIGS. 5 and 6. Eggs from the uterus showing normal position of ovum and its relation to yolk cells. Longitudinal sections.

FIG. 7. Young egg showing terminal position of ovum and arrangement of shell material. Longitudinal section.

FIG. 8. Egg in longitudinal section.

FIGS. 9 and 10. Eggs from terminal portion of uterus showing abnormal position of ovum. Transverse and longitudinal sections.

FIGS. 11, 12 and 13. Eggs in transverse section.

FIG. 14. Egg showing position of vitelline membrane before (in dotted line) and after escape of the miracidium in hatching, living specimen.

FIG. 15. Miracidium showing excretory system and arrangement of ciliated epidermal cells, living specimen.

FIG. 16. Miracidium showing ganglionic mass and germ cells, living specimen.

FIG. 17. Metacercaria emerging from the cyst, mounted specimen.

FIG. 18. Metacercaria in transverse section.

FIG. 19. Excretory bladder of the adult worm showing protuberances of the wall. Detail from section.

FIG. 20. Metacercaria five weeks after infection of the crayfish, living specimen.

FIG. 21. Mature encysted metacercaria, living specimen.

FIG. 22. Mature excysted metacercaria showing excystation glands and genital primordia. Composite drawing from living and mounted specimens.

FIG. 23. Mature excysted metacercaria showing the grosser portion of the excretory system and the vacuolate bodies situated along the edge of the ceca, living specimen.

FIG. 24. Young adult worm from the lung of a cat 31 days after infection, mounted specimen.

PLATE II.

All original drawings were made with the aid of a camera lucida and with the exception of figs. 38, 41, 42 and 43 were made from preserved and mounted specimens. Figures 38, 41, 42 and 43 were drawn from living specimens.

FIGS. 25 and 26. Young sporocysts. Removed from snail 14 days after penetration of miracidia.

FIG. 27. Mature sporocyst containing well developed first generation rediae. Removed from snail 39 days after infection.

FIGS. 28 and 29. Mature first generation rediae containing well developed second generation rediae. Removed from snail 114 days after infection.

FIGS. 30 and 31. Young second generation rediae. Removed from snail 124 days after infection.

FIGS. 32 and 33. Smallest and largest pharynges of mounted second generation rediae containing large, well developed cercariae. From a naturally infected snail. Figures 28-33 inclusive have the same scale.

FIGS. 34 and 35. Young first generation rediae. Removed from snail 28 days after penetration of miracidia. At same scale.

FIG. 36. Young first generation redia. Removed from snail 78 days after infection.

FIG. 37. Reproduction of tracing of photograph of second generation rediae by Ando (1919).

FIG. 38. Gut of second generation redia showing cell structure.

FIG. 39. Second generation redia, after Nakagawa (1919).

FIG. 40. Second generation redia, after Kobayashi (1921).

FIG. 41. Mature second generation redia from a naturally infected snail.

FIG. 42. A normally emerged cercaria.

FIG. 43. Immature cercaria removed from the snail.

FIG. 44. Cercaria after Kobayashi (1921).

FIG. 45. Cercaria after Nakagawa (1919).

PLATE III.

Spines from various hosts. Drawn to same scale with the aid of a camera lucida.

FIG. 46. From metacercaria, between oral sucker and acetabulum.

FIG. 47. From between oral sucker and acetabulum of a worm 12 days in a white rat.

FIG. 48. From worm 20 days in white rat, from the lungs.

FIG. 49. Apical view of spines from ventral surface, testicular region of worm from lungs of white rat 106 days after infection.

FIG. 50. From posterior dorsal surface of above worm.

FIG. 51. From posterior dorsal surface of a worm from the lungs of a cat 35 days after infection.

FIG. 52. From posterior ventral surface of a worm from the lungs of a cat 42 days after infection.

FIG. 53. From between oral sucker and acetabulum of a worm from the lungs of a cat 35 days after infection.

FIG. 54. From posterior dorsal surface of a full grown worm from a cat.

FIG. 55. From posterior ventral surface of a worm from the lungs of a white rat 106 days after infection.

FIG. 56. From ventral surface, testicular region of above worm.

FIG. 57. From middle dorsal surface of a full grown worm from a cat.

FIG. 58. From ventral surface, testicular region of a full grown worm from a cat.

FIG. 59. From ventral surface, testicular region of same worm.

FIG. 60. From ventral surface, testicular region of a full grown worm from a cat.

FIG. 61. From posterior ventral surface of above worm.

FIG. 62. Spines from above worm, shattered by pressure.

FIGS. 63 and 64. From region adjacent to posterior edge of acetabulum.

FIG. 65. From posterior ventral surface of a full grown worm from a pig.

FIG. 66. From middle dorsal surface of a full grown worm from a cat.

FIG. 67. From ventral surface, testicular region of a full grown worm from a mink.

FIG. 68. From posterior dorsal surface of above worm.

PLATE IV.

Spines from various hosts. Drawn to the same scale with the aid of a camera lucida.

FIG. 69. From middle dorsal surface of a full grown worm from a human in Korea.

FIG. 70. From ventral surface, testicular region of same worm.

FIG. 71. From between oral sucker and acetabulum of same worm.

FIG. 72. From anterior dorsal surface of same worm.

FIG. 73. From posterior ventral surface of same worm.

PLATE I.



PLATE II.

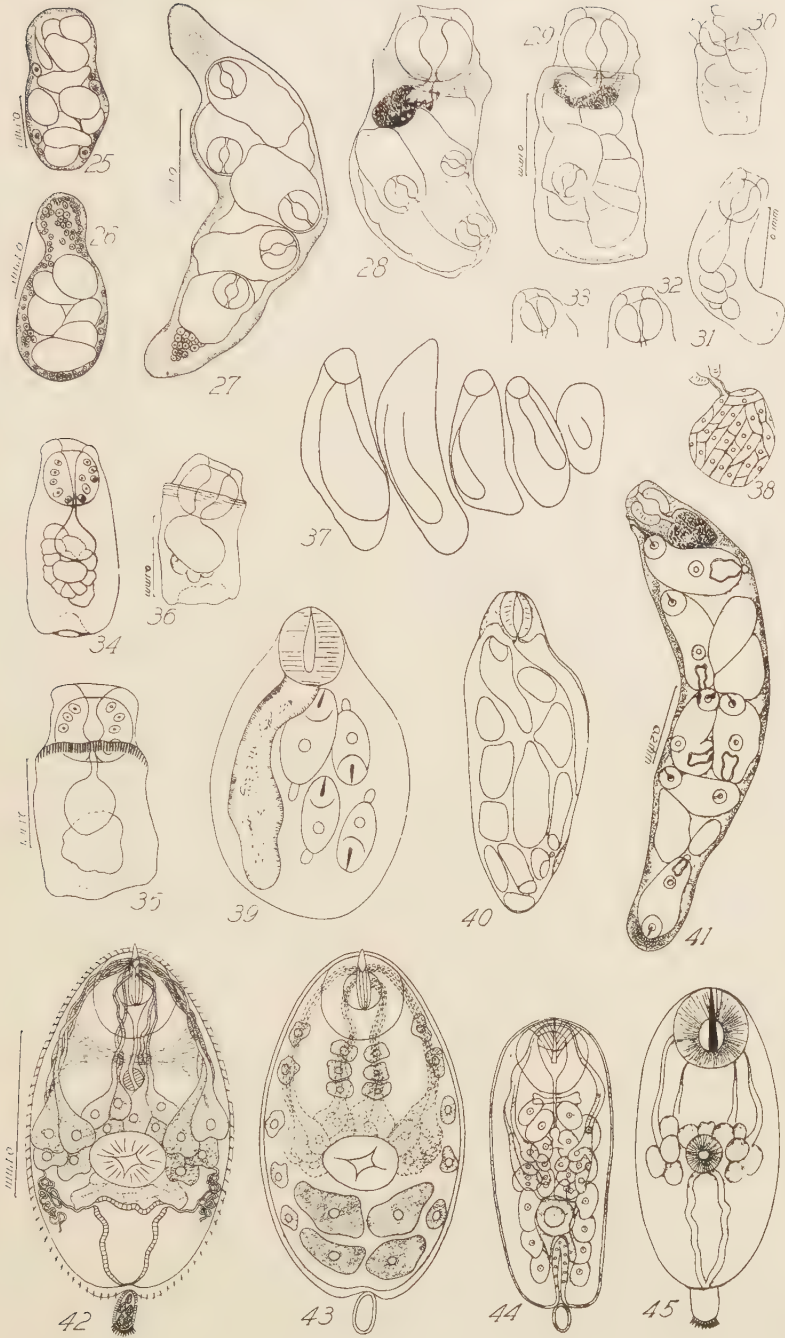


PLATE III.



PLATE IV.



PLATE VA.

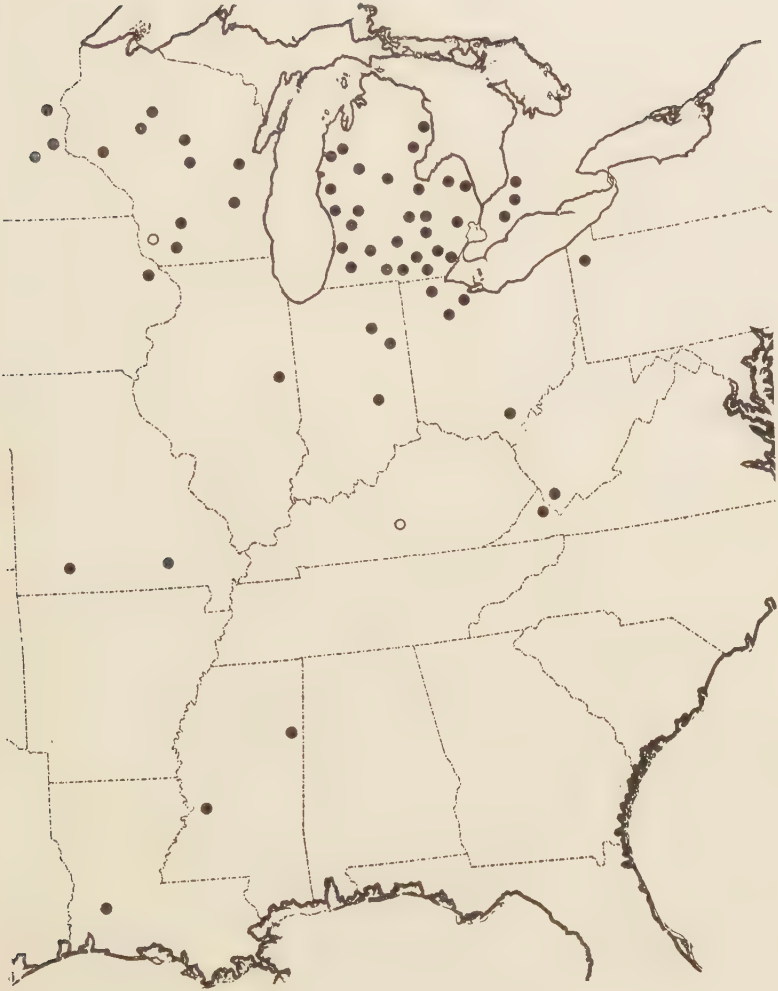


PLATE VB.



92



93



94



95



96

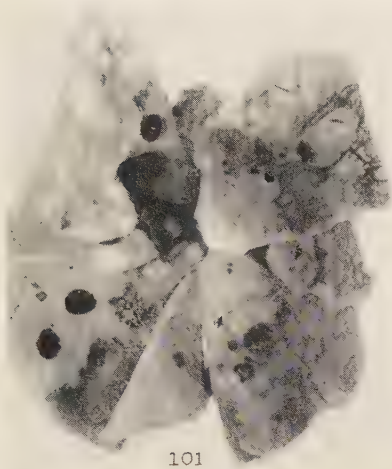


97



98

PLATE VI.



101



99



100

FIG. 74. From middle dorsal surface of a full grown worm from a dog in Korea.

FIG. 75. From posterior ventral surface of same worm.

FIG. 76. From between oral sucker and acetabulum of same worm.

FIG. 77. From ventral surface, testicular region of same worm.

FIG. 78. From lateral cuticle of a worm mounted by Hirsch and labeled *P. ringeri*.

FIGS. 79-81. Apical view of spines from the ventral cuticle of a worm mounted by Hirsch and labeled *P. ringeri*.

FIGS. 82 and 83. Spines drawn from the above mount.

FIG. 84. From middle dorsal surface of a full grown worm from a Malayan tiger.

FIG. 85. From between oral sucker and acetabulum of above worm. Cuticle subjected to pressure.

FIG. 86. From ventral surface, testicular region of above worm.

FIG. 87. From posterior ventral surface of a full grown worm from a Sumatran tiger.

FIG. 88. From posterior ventral surface of a full grown worm from a Malayan tiger.

FIG. 89. From ventral surface, testicular region of a full grown worm from a Sumatran tiger.

FIG. 90. From between oral sucker and acetabulum of above worm.

PLATES VA AND VB.

FIG. 91. Map showing distribution of new records of *Paragonimus*. The Minnesota records were made by Wallace and the open circles indicate those by Dearborn. The remaining records were made by the writer.

FIGS. 92-96. Camera lucida drawings of mounted mature sporocysts to show the various shapes they may assume.

FIGS. 97-98. Camera lucida drawings of spent sporocysts.

PLATE VI.

FIG. 99. Specimens of *Pomatiopsis lapidaria*. About $\times 1\frac{1}{2}$.

FIG. 100. Metacercariae in the heart of a crayfish.

FIG. 101. The lungs of an infected cat showing the cysts of the lung fluke.

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